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(71) Applicant: MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK [US/US]; One Gustave L. Levy Place, New York, NY 10029-6574 (US).

(72) Inventors: CONSTANTIN, Bona ; 333 E. 55th Street, New York, NY 10022 (US). HABIB, Zaghouani ; 150 E. 85th Street, Apt. 7A, New York, NY 10028 (US).

(74) Agent: CLARK, Richard, S.; Brumbaugh, Graves, Donohue & Raymond, 30 Rockefeller Plaza, New York, NY 10112 (US).

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(54) Title: ANTI-HUMAN IMMUNODEFICIENCY VIRUS RECOMBINANT ANTIBODIES

(57) Abstract

This invention provides hybrid antibodies which comprise a base portion which corresponds to the constant portion of human immunoglobulin G, a combining site selected for specificity to the particular target antigen, and a combining site which binds to and activates human CTL.

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Description

Anti-Human Immunodeficiency Virus Recombinant Antibodies

Background of the Invention

This invention relates to recombinant hybrid molecules for use in therapy and prevention of viral infections.

There are a wide variety of foreign substances or organisms which can enter the body to cause illness. Mammals including man respond to such an invasion with an "immune response" which is the result of many complex interactions between a variety of cells and humoral factors. Although many different cells participate, lymphocytes are the primary cells involved in generating an immune response so as to protect an individual from foreign substances such as bacteria, viruses and foreign cells.

There are two principal classes of lymphocytes, B cells and T cells. Both classes are derived from progenitor hematopoietic stem cells. Mature T cells have been classified into three subpopulations based on the different tasks they perform. Helper T cells (T_h) are required for promoting or enhancing B cell antibody production. Cytotoxic killer T cells (T_k), otherwise known as cytotoxic T lymphocytes (CTL) directly kill their target cells by cell lysis. Suppressor T cells (T_s) suppress or down-regulate immunological reactions.

These different subpopulations of T cells express a variety of cell surface proteins some of which are termed "marker proteins" because they are characteristic of the particular subpopulations. For example, most of the T_h cells express the cell surface CD4 protein, whereas most CTL and T_s cells express the

cell surface CD8 protein. Swain, "Evidence for two Distinct Classes of Murine B Cell Growth Factors with Activities in Different Functional Assays", J. Exp. Med., 158:822 (1983). Additionally, mature T cells can be distinguished from immature T cells (thymocytes) by the presence of the cell surface T cell receptor (TCR), a transmembrane protein complex found on mature T cells which is capable of recognizing antigen in association with self-antigens encoded by MHC genes.

As it is now understood, initiation and maintenance of immune responses involve cell to cell interactions and depend on the recognition of and interactions between particular proteins or protein complexes on the surface of B cells, T cells, foreign substances, foreign cells and infected cells.

There are at least two separable aspects of the immune response, cell-mediated and antibody-mediated responses. Both begin when a T cell recognizes a foreign antigen. The cell-mediated response involves the lytic activity of CTL activated by exposure to antigen and proceeds in the absence of B cells. CTL can also be nonspecifically activated to lyse any cell in close proximity by having an antibody bound to a cell-surface protein such as CD3. For the antibody-mediated response to occur, the T_h cell which has been activated by exposure to a foreign antigen interacts with a B cell to stimulate B cell production of humoral proteins known as immunoglobulins or antibodies.

Although T cells directly participate in the cell-mediated immune responses to foreign antigens, B cell production of antibodies is the most important aspect of immunity. The requisite variety of antibodies is provided by the diversity of immunoglobulin genes. Genetic rearrangement further increases their variety. Each set of mature

immunoglobulin genes is the result of a further genetic rearrangement. Providing yet more diversity, there are several immunoglobulin classes with varying features. For a review of immunoglobulin genetics and protein structure see Lewin, "Genes III", John Wiley and Sons, N.Y. (1987).

The developing techniques of genetic engineering have been employed in various approaches to assist the natural immune system and to provide reagents for performing diagnostic tests. For instance, protein sequences corresponding to the antigenic determinants of various organisms suitable for use as vaccines have been prepared both synthetically and by recombinant DNA techniques.

Antibodies are extremely important in diagnostic and therapeutic applications due to their diversity and specificity. Molecular biology techniques have been used to increase the availability of antibodies for scientific applications. For instance, a single antibody producing B cell can be immortalized and expanded to provide an in vitro source of antibodies of a single specificity known as a "monoclonal antibody" (mAb). Such an immortal B cell line is termed a "hybridoma".

Until recently, the source of most mAb has been murine (mouse) hybridomas. Although they have been used extensively in diagnostic procedures, murine mAb are not well suited for induction of passive immunity or other therapeutic applications in mammals including humans and nonsyngeneic mice. Moreover, murine antibodies are recognized as foreign by other mammalian species and elicit an immune response which may itself cause illness. Human mAb would therefore be extremely useful in the treatm nt of a wide variety of human diseases. However, production of human mAb has proven to be much more difficult than that of murine mAb.

Consequently they are not yet available in sufficient quantities or varieties to be used as therapeutics.

To overcome the problems of immune responses to foreign mAb and the lack of suitable human mAb, at least in part, genetic engineering techniques have been used to construct hybrid immunoglobulin molecules which contain the antigen binding region of the murine antibodies and the remainder of the molecule is composed of human antibody sequences which are not recognized as foreign. Jones et al., "Replacing the Complementarity-Determining Regions in a Human Antibody With Those From a Mouse", Nature, 321:522-525 (1986). These hybrid antibodies eventually elicit an immune response in human therapy, and they often do not function as effectively as the parent murine antibodies. For a review of the use and drawbacks of murine and human mAb see Carlsson et al. "Monoclonal Antibodies into the 90's: the All Purpose Tool", Bio/Technology, 7:567-573, (1989).

Summary of the Invention

The present invention provides novel therapeutic agents and methods which combine the cell-mediated and antibody-mediated aspects of the immune response in a single agent for use in human therapy, particularly viral therapy and in tumor therapy. In therapy of viral infections, these novel agents act to block virus infection by focusing cytotoxic T lymphocytes (CTL) to virally infected cells thus causing lysis of the infected cells.

This invention provides hybrid antibodies which comprise a base portion corresponding to the constant portion of human immunoglobulin G, a combining site selected for specificity to the particular target antigen, and a combining site which binds to and activates human CTL. If the hybrid antibodies are to

be used in therapy of viral infections, preferred hybrids have a target antigen combining site which binds to the protein responsible for virus infection, the viral "cell-recognition" protein, thus neutralizing infectivity of the virus. Since the cell-recognition protein is often expressed on the surface of virally infected cells, the hybrid antibody can also bind to these infected cells. Binding of the hybrid antibodies to both a virus infected cell and CTL causes activation of the CTL and subsequent lysis of the infected cell.

A hybrid antibody according to the invention for treatment of infections by human immunodeficiency virus (HIV), which causes the acquired immune deficiency syndrome (AIDS) disease, preferably includes a combining site which binds to the protein CD3 so as to activate CTL, and a combining site specific for both HIV antigens budding from the surface of infected cells. For example, the antigen-recognition combining site might be the variable portions of an antibody specific for HIV coat proteins.

Brief Description of the Drawings

Figure 1 is an illustration of an immunoglobulin molecule illustrating its Y shape, combining sites, hinge regions, light and heavy chains and their corresponding variable and constant domains.

Figure 2 is an illustration of a protein complex containing a single immunoglobulin combining site capable of recognizing a virus, virus infected cell or viral antigen, a single immunoglobulin combining site capable of recognizing and binding to CD3 so as to activate CTL, an immunoglobulin hinge region separating the combining sites from the immunoglobulin constant domain CH2 and CH3 regions.

Figure 3 is a schematic diagram of a DNA construct containing the VH-D-J gene.

Figure 4 is a flow diagram showing a cloning scheme of the VH-D-J region.

Detailed Description of the Invention

It has now been found that the antibody-mediated and the cell-mediated immune responses can be combined in a single recombinant protein complex so as to offer novel therapeutic advantages for diseases such as viral infections. The invention relates to hybrid antibodies engineered by recombinant DNA techniques which are useful in therapy and prevention of viral infections in humans.

Central to the hybrid antibody of the invention is a base portion comprising at least a part of human immunoglobulin G (IgG). As shown schematically in Figure 1, IgG is a tetrameric protein complex formed from two identical heavy chains H and H′ and two identical light chains L and L′. These chains are joined by disulfide bonds into a Y-shaped complex. In solution however, the molecule takes on a more globular shape.

Protein sequence analysis of immunoglobulins has led to the definition of specific regions or functional domains within each of these chains. Each chain has a variable region (VL and VH) located at its amino terminus. The variable domains created by the pairing of the VL and VH regions constitute the antigen-recognition portion or "combining site" of the molecule. There are two combining sites per molecule. The variable domains of these chains are highly variable in sequence and provide the diversity for antibody combining sites to be highly specific for a variety of antigens. Each of the chains also includes essentially constant regions, which do not vary in response to the nature of the antigen recognized by the combining sites. The light chains have a single

constant region (CL), while the heavy chains possess three separate constant regions (CH1, CH2 and CH3). The pairing of CL and CH1 produce the first constant domains, C1, while the pairing of the CH2 regions produces the second constant domain, C2 and the pairing of the CH3 regions produces the third constant domain, C3. The four constant domains, two C1's, C2 and C3, constitute the Y shaped base portion of the immunoglobulin molecule. In addition, the heavy chains also have a hinge region separating C1 and C2 from the remainder of the molecule. The hinge imparts flexibility to the tetramer.

In a preferred embodiment, the protein complexes of the invention have a Y shaped base portion which is the same as some or all of the constant regions of human immunoglobulin. This use of human immunoglobulin avoids the problem of the modified immunoglobulin being recognized as a foreign species itself, and thus facilitates its use in human therapy. Additionally the base portion may confer effector functions on the molecule such as <u>in vivo</u> stability, Fc receptor binding, protein A binding, complement fixation, and placental transfer. It will thus be understood that modified sequences based on immunoglobulin molecules are within the scope of the present invention so long as the modification does not give rise to immune rejection problems.

To the base portion, there is added a combining site which binds to and activates CTL, and a combining site which binds to antigen. A particularly suitable antibody combining site for CTL activation is the combining site of an antibody specific to the CTL cell surface protein CD3. Antibodies specific to other CTL surface proteins which also function to activate CTL are also encompassed within the scope of this invention. These combining sites are affixed via

peptide bonds to the amino terminal ends of the base portion on the arms of the immunoglobulin-like Y.

The antigen-recognition combining site is selected to provide specificity to a particular target organism. For example, the combining site of an antibody specific to the target organism can be affixed to the amino terminal end of an arm of the base portion.

In the preferred embodiment of the present invention, the antigen-recognition combining site is affixed via peptide bonds to one arm of the Y shaped base portion and the antibody combining site specific for CTL is affixed via peptide bonds to the other arm of the Y shaped base portion.

The hybrid immunoglobulins of the present invention are useful in the treatment of a wide variety of viral infections. They are particularly well suited for treatment of infections by viruses which upon infection of the host cell cause expression of viral coat proteins prior to cell death. In most cases this cellular expression of viral coat proteins leads to a cell surface form of such proteins. Examples include but are not limited to the hemagglutinin protein complex of influenza virus, the env proteins of murine leukemia virus, the env proteins of Rous sarcoma virus and the env proteins of HIV. Often the viral protein expressed by infected cells is the same viral coat protein which recognizes and binds to the cell receptor protein to initiate infection. This is true in the case of HIV.

It is well known that anti-idiotype antibodies carrying the internal image of microbial antigens as well as antibodies against TCR of T cells can stimulate humoral and cellular antimicrobial immunity.

A preferred embodiment to create such novel antibodies is to incorporate antigenic sequences directly into the antibody by genetic manipulation. A

method is described herein whereby such antibodies are produced by genetic engineering to replace a segment of immunoglobulin molecule with a sequence corresponding to HIV antigenic determinants recognized by B or T cells.

Exemplifying the present invention, the D segment of the heavy chain of an antibody has now been replaced by influenza virus nucleoprotein (NP) epitope which is capable of being recognized by T cells. The construct was expressed in the SP2/0 myeloma cell line. Such transfected SP2/0 were killed by T cells specific for the NP epitope.

In the Examples provided below, two DNA expression vectors pSV2gpt-91A3VH-CIgG2b and pSV2neo-91A3L, both carrying a heavy and a light chain gene of an anti-arsenate antibody called 91A3. The pSV2gpt-91A3VH-CIgG2b carries an IgG2b constant region gene inserted in the HindIII restriction endonuclease site and the rearranged 5.5 kb VHDJ gene of the 91A3 antibody inserted in the EcoRI restriction endonuclease site as shown in Figure 3. The 5.5 kb fragment also contains the heavy chain Ig promoter and enhancer. The pSV2neo-91A3L carries the rearranged VL and CL genes and the necessary regulatory elements inserted into the EcoRI and BamHI restriction endonuclease sites. It has now been shown that cotransfection of these vectors into the nonsecreting myeloma cell line, SP2/0 leads to the expression of a functional 91A3 antibody.

This antibody derives its VH from the J558 family and its D segment is probably involved in antigen binding. These observations suggest that these D segments are surface exposed. In fact, the hydrophilicity profile of the 91A3 VH also predicts that its D segment is surface exposed. For these reasons the 91A3VHDJ was chosen to construct the Ig chimera carrying the NP epitope. The goal of this

study was to replace the 9 amino acid D segment with a 15 amino acid NP CTL epitope as illustrated in Figure 3.

This epitope corresponds to amino acid residues 147-161 within the NP of PR8 virus and is known to induce virus specific CTLs in Balb/C but not C57BL/6 mice.

The molecules of the present invention are the product of recombinant DNA engineering or chemical cross-linking. Methods of fusing genes in the proper orientation, transforming the genes into a suitable host cell and expressing and purifying the proteins are known in the art and examples are provided below. Detailed DNA cloning methods are provided in a variety of sources. See e.g. Sambrook et al., "Molecular Cloning A Laboratory Manual", Cold Spring Harbor Laboratory Press, NY (1989).

Once the fused genes have been cloned, they are transfected into a suitable host for expression of the encoded protein. The cloned gene may be first inserted into an appropriate expression vector or may be transfected into the cell as linear DNA for recombination with the host genome. Suitable expression vectors include but are not limited to plasmids, viruses and retroviruses. Choice of a suitable vector will be determined in part on the choice of the host used for protein expression. Suitable hosts include but are not limited to bacteria, mammalian cell lines, whole animals such as transgenic mice and insect cell lines. Although insect cell lines have not heretofore been used for the expression of immunoglobulin proteins it is thought that the difference in glycoprotein patterns compared to the products of mammalian cell lines may produce more effective proteins. Insect cell lines are less expensive to maintain and produce more protein compared

to mammalian cell lines and are thus more suitable to large-scale protein production. Genes expressed by insect cell lines do not contain exons therefore the exons should be excised in genes prior to their expression in insect cell lines. Excision is relatively straightforward and can be accomplished for instance directly by oligonucleotide directed site-specific mutagenesis or indirectly by cDNA cloning.

Transfer of the gene into the host can be done by any of the well known means in the art. For example, methods of gene transfer include but are not limited to $CaCl_2$ mediated transfection in the case of bacteria and in the case of eukaryotic cells, $CaPO_4$ mediated transfection, viral infection including retroviral latent infection, electroporation, liposome mediated DNA transfer and microinjection among others.

Any suitable method of purifying proteins produced by the host may be used in the practice of the present invention. See e.g. Webb et al., "Cell-surface Expression and Purification of Human CD4 Produced in Baculovirus-infected Insect Cells", Proc. Natl. Acad. Sci. USA, 85:7731-7735 (1989); and Moran et al., "Characterization of Variable-Region Genes and Shared Crossreactive Idiotypes of Antibodies Specific for Antigens of Various Influenza Viruses", Vir. Immunol., 1:1-12 (1987).

The present invention is useful in directing the cell-mediated immune response against virally infected cells. HIV infected cells are used here as an example of the utility of the present invention but it should be understood that other diseases could be treated and are considered to be within the scope of the invention.

As with all pharmaceutical compositions, the effective amounts of the antibodies of the invention must be determined empirically. Factors to be

considered include the condition to be treated, whether or not the antibody will be complexed with or covalently attached to a toxin, route of administration for the composition, i.e. intravenous, intramuscular, subcutaneous, etc., and the number of doses to be administered. Such factors are known in the art and it is well within the skill of physicians to make such determinations without undue experimentation.

The following examples are meant to illustrate but not limit this invention.

Example 1 - DNA Cloning

The procedure for deleting the 27 nucleotides coding for the D segment of IgG, and the insertion of 45 bases corresponding to the NP epitope, is summarized in Fig. 4. All enzymes were used according to the manufacturer's instructions (New England Biolabs, Beverly, MA). Unless otherwise specifically mentioned, DNA cloning was performed according to the methods described in Maniatis et al. (1982).

Using this method the D segment of VH region of 91A3 anti-arsonate antibody is replaced with one of:

(a) The consensus sequence of the B cell epitope of the cysteine loop of gp120. The sequence of this epitope varies, however, a consensus sequence deduced from 245 HIV isolate sequences borne by 241 isolates was established. The amino acid sequence of the consensus corresponds to residues 301-319 of gp120 and is as follows:

Arg-Lys-Ser-Ile-His-Ile-Gly-Pro-Gly-Arg-Ala-Phe-Tyr-Thr-Thr-Gly-Glu-Ile-Ile

(b) The T cell epitope of residues 12-35 of gag of HIV-1 HxB2 isolate:

Glu-Leu-Asp-Arg-Trp-Glu-Lys-Ile-Arg-Leu-Arg-Pro-Gly-Gly-Lys-Lys-Lys-Tyr-Lys-Leu-Lys-His-Ile-Val

(c) A T cell epitope of HIV-1 reverse transcriptase; residues 325-349

Ala-Ile-Phe-Gln-Ser-Ser-Met-Thr-Lys-Ile-Leu-Glu-Pro-Phe-Arg-Lys-Gln-Asn-Pro-Asp-Ile-Val-Ile-Tyr-Gln

Briefly, cloning was done by subcloning the 5.5 kb 91A3VHDJ fragment into the EcoRI restriction endonuclease site of the pUC19 plasmid. Two unique restriction endonuclease sites (NcoI and ApaI, 638 bp apart) surrounding the D region were identified. The primers P1 and P3, shown in Fig. 4, are exactly complementary to their corresponding strands. However P2 matches with its complementary strand down to the last nucleotide 5′ of the D region (filled part of the bar). The remaining 30 nucleotides (hatched part of the bar) are those of the NP epitope. Primer P4 contains nucleotides complementary to the corresponding strand down to the last nucleotide 5′ of the D region. The remaining unmatched nucleotides correspond to 30 bases of the NP epitope. An SpeI restriction endonuclease site was created within the overlapping nucleotides between P2 and P4.

Using polymerase chain reaction, two fragments are produced. In one set of reactions, the annealing of the P3 and P4 primers to the plasmid results in the production of 570 bp fragment. In another set of reactions, the annealing of P1 and P2 to plasmid provides a 326 bp fragment. To delete the NP overlapping sequences, both fragments are digested with SpeI. The ligation of fragments, sharing each half of the NP epitope, generates an 870 bp fragment containing the 45 bp NP epitope inserted in-frame. The following steps consist of digesting both the original pUC19-VHDJ91A3 and the 870 bp fragment with the restriction endonucleases NcoI and ApaI. The ligation of the 656 bp fragment into the digested plasmid provides a vector possessing the coding region of the NP epitope instead of the D segment. The 5.5 kb EcoRI

VH-NP.J fragment is then subcloned into the EcoRI restriction endonuclease site of the expression vector.

Cotransfection was done using the gene pulsar transfection apparatus according to the manufacturer's instructions (Biorad). Cotransfection of the plasmid pSV2gpt-91A3-VHNPJ-CIgG2b and the pSV2neo-91A3L plasmid into the non-secreting myeloma cell line SP2/0 and selection with mycophenolic acid and geneticin (G418) allows the synthesis and secretion of the 91A3-NP chimeric antibody.

SP2/0 are contransfected with heavy chain bearing HIV epitopes together with parental light chain to create transfectomas. Antibodies produced by these transfectomas are used to induce humoral or cellular anti-HIV immunity.

Example 2 - Activity of Chimeric Antibodies

NP-specific cytotoxic T cell clones have been generated from Balb/c mice immunized with PR8 influenza virus and expanded in vitro with irradiated spleen cells coated with 5 μg NP. The cytotoxicity assay was carried out by incubating ^{51}Cr-labeled target cells and NP-specific CTL at 10:1 E/T ratio for 4 hours. The coating of target cells with NP was performed by incubating 10^6 cells with 5 μg peptide for 30 minutes, washing and then labeling with ^{51}Cr as previously described by Ito et al., J. Immunol. Met., 103:229 (1987). NP peptide (TYQRTRALVRTGMDP) is a T cell epitope recognized in association with H-$2K^d$ whereas the peptide (IASNENMDAMESSTS) is a T cell epitope recognized in association with H-$2D^b$ antigen.

The results presented in Tables 1 and 2 show that chimeric Ig bearing the influenza virus epitope bound a rabbit anti-NP antibodies and lost its binding to arsonate since the D segment which plays an important role in the binding of arsonate was replaced with viral peptide.

Table 1

Immunochemical Properties of Immunoglobulins Produced by SP2/0 Coinfected with pSV2gpt-91A3gpt-91A3V_H and pSV2neo-91A3L

Binding to	Binding of 91A3Ig produced by T14-10 (in cpm)
Ars BSA	15,445 ± 101
Rabbit Antimouse IgG_2b	42,724 ± 127

Binding to arsonate was determined by incubation of 10ng of antibody on a microtiter plate coated with either arsonate BSA or BSA alone and bound antibodies were revealed with ^{125}I rat antimouse κ antibody. Binding to anti-isotype antibody was performed by incubation of 10ng of antibodies on plates coated with rat antimouse κ mAB and bound antibody was revealed using ^{125}I goat antimouse IgG2b antibodies.

Table 2

Binding Properties of 91A3 Chimeric Immunoglobulin (in cpm)

Binding to	91A3-NP (chimeric)	91A3 (native)
Arsonate BSA	792 ± 22	15,445 ± 101
Anti-NP antibodies	5,616 ± 217	1,246 ± 76

Binding to arsonate-BSA was carried out as previously described in Example 1. Binding to rabbit anti-NP antibodies was assessed by incubating transfectoma supernatants on microtiter plates coated with affinity chromatography purified anti-NP antibodies and bound antibodies were revealed using ^{125}I goat antimouse IgG2b.

NP-specific CTL were able to kill SP2/0 transfected with chimeric Ig gene indicating that NP

epitope is expressed on cell-surface as in cells infected with the virus.

The data in Table 3 (panel A) show that the CTL clone is able to kill PR8 and X31 influenza virus infected P815 cells (H-2^d) as well as P815 cells coated with NP. No significant killing was seen with P815 cells coated with irrelevant NP known to be recognized in association with H-$2D^b$ by C57BL/6 CTL. Panel B shows the ability of NP specific CTL to kill SP2/0 cells, expressing chimeric Ig genes, or coated with NP. No killing was observed with cells expressing V_{HW}, V_{LW} or both genes. However, significant killing is observed with SP2/0 V_{HC}-V_{LW} transfectomas.

Table 3

Killing of SP2/0 cells transfected with plasmid carrying the V_H-NP chimeric gene (V_{HC}), by NP-specific CTL

	Target Cells	% Specific (1)	^{51}Cr release (2)
A	P815	14	12
	P815-NP H-2^d	77	49
	P815-NP H-2^d	14	10
	P815 infected with PR8	59	51
	P815 infected with X31	77	64
	P815 infected with B Lee	19	9
B	SP2/0	ND*	2
	SP2/0 / V_{HW}-V_{LW}	9	ND
	SP2/0 / V_{HW}-V_{LW} coated with NP-H2^d	30	ND
	SP2/0 / V_{HW}-V_{LW} coated with NP-H2^b	7	ND
	SP2/0 / V_{HW}	2	ND
	SP2/0 / V_{LW}	4	ND
	SP2/0 / V_{HC}	44	39
	SP2/0 / V_{HC}-V_{LW}	28	21

* ND = not done

These results clearly show that cells transfected with chimeric immunoglobulin genes bearing an epitope of influenza virus recognized by CTL are killed by CTL as are influenza infected cells or cells artificially (in vitro) coated with peptide.

Claims

1. A hybrid antibody comprising
   (a) a base portion which comprises at least one of two constant domains (CH2 and CH3), a hinge region associated via disulfide bonds in the same relation as in the Y-shaped structure which is characteristic of human immunoglobulin G;
   (b) a first combining site connected by peptide bonds to the base portion said first combining site being capable of forming a bond between the hybrid immunoglobulin and human cytolytic T lymphocyte cells, thereby activating the cytolytic T lymphocyte cells; and
   (c) a second combining site which is not the same as the first combining site, connected by peptide bonds to the base portion.

2. A hybrid immunoglobulin according to claim 1 wherein the first combining site specifically binds to CD3.

3. A hybrid immunoglobulin according to claim 1 wherein the second combining site specifically binds to a human immunodeficiency virus coat protein.

4. A chimeric immunoglobulin bearing a viral epitope to elicit a humoral and cell mediated anti-viral immune response.

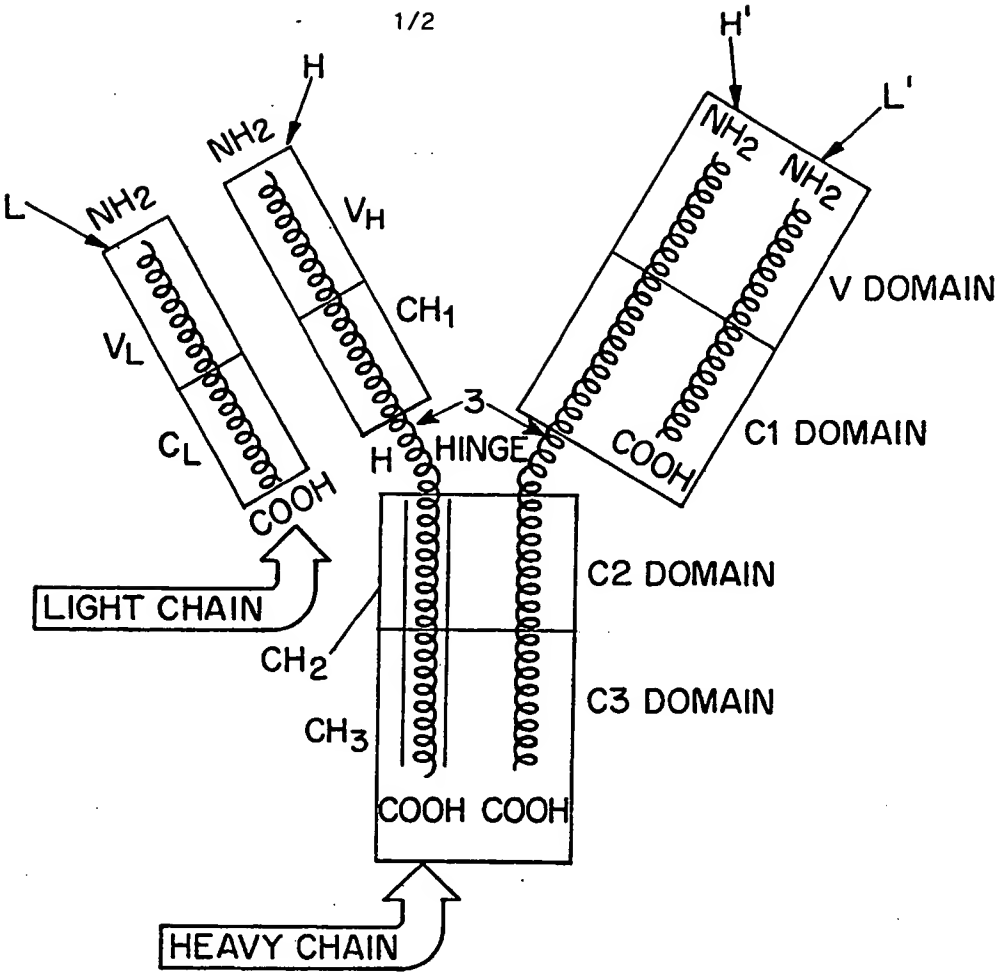
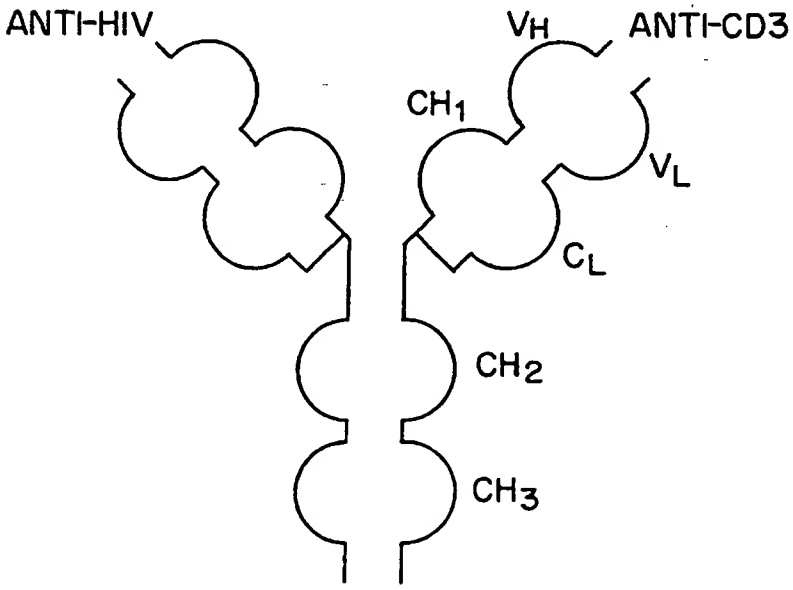
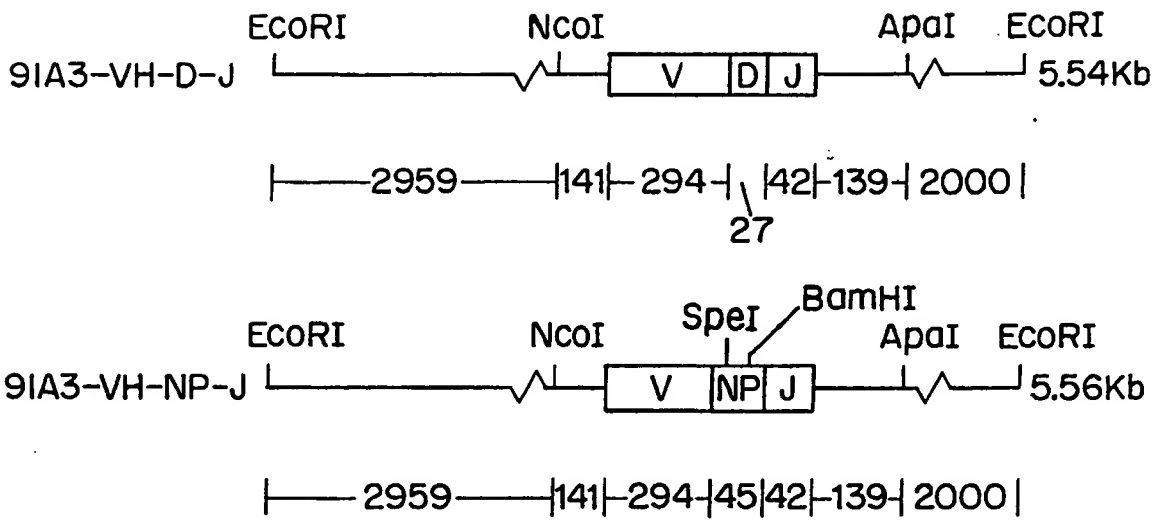


FIG. 1



HYBRID CD4 ANTI-CD3 MOLECULE

FIG. 2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2000
27
EcoRI
NcoI
SpeI
BamHI
ApaI
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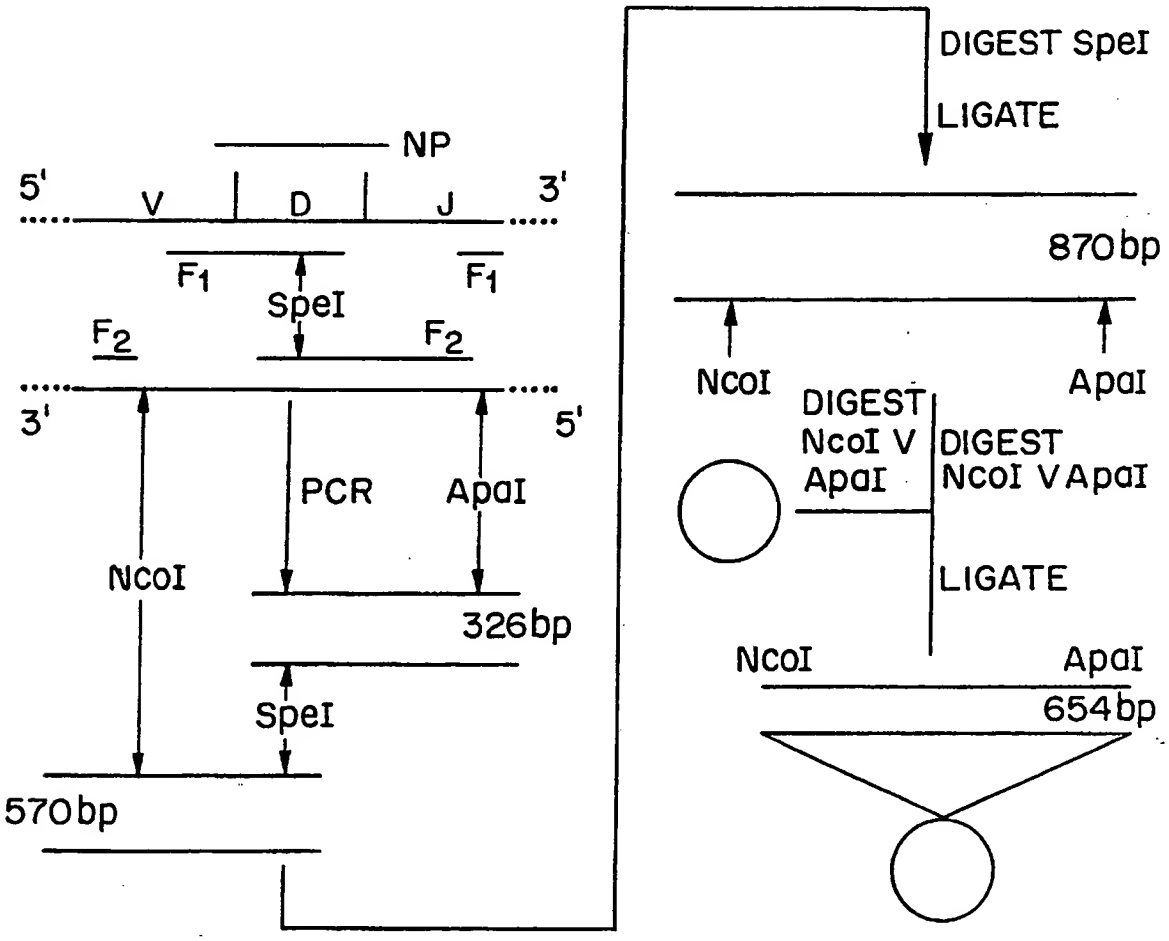
141
294
45
42
139
2000

FIG. 3


NP
5'
V
D
J
3'
F1
F1
SpeI
F2
F2
3'
5'
PCR
ApaI
NcoI
326bp
SpeI
570bp
DIGEST SpeI
LIGATE
870bp
NcoI
ApaI
DIGEST
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NcoI
ApaI
654bp

FIG. 4

I. RNATIONAL SEARCH REPORT

International application No.
PCT/US92/02975

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C07K 15/28
US CL :530/387.3, 388.35
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U.S. : 530/387.3, 388.35
C07K 15/28

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, MEDLINE, BIOSIS, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal of Immunological Methods, Volume 129, issued 1990, Moran et. al. " A Novel Technique for the Production of Hybrid Antibodies", Pages 199-205, see entire document.	1
X	Journal of Immunology, Volume 146, No. 1, issued 01 January 1991, Moran et. al., "Inhibition of Multicycle Influenza Virus Replication by Hybrid Antibody-Directed Cytotoxic T lymphocytic Lysis", Pages 321-326, see pages 321-324.	1
Y	EP 0,308,936 (Zarling et al.) 28 March 1989, see entire document.	2 and 3
Y	Mount Sinai Journal of Medicine, Volume 53, No. 3, issued March 1986, Morrison et. al. "Production of Novel Immunoglobulin Molecules by Gene Transfection", Pages 175-180, see entire document.	4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
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